Reference: *Biol. Bull.* **169:** 342–364. (October, 1985)

# OVULATION AND THE FINE STRUCTURE OF THE *STICHOPUS CALIFORNICUS* (ECHINODERMATA: HOLOTHUROIDEA) FECUND OVARIAN TUBULES

S. SMILEY AND R. A. CLONEY

*Zoology Department, University of Washington, Seattle, Washington 98195 and Friday Harbor Laboratories, 620 University Road, Friday Harbor, Washington 98250*

## ABSTRACT

The ovary of *Stichopus californicus* consists of several size classes of tubules, which insert into a central gonad basis. The largest tubules contain the oocytes that will be spawned in the current season. All tubules are composed of three layers. Outermost is a complex peritoneum composed of epithelial cells, axons and muscle cells. The fine structure of the peritoneal neurons suggests their involvement in neurosecretory activity. Between the basal laminae of the peritoneum and the inner epithelium is the ovarian connective tissue compartment, including the genital hemal sinus. This sinus probably conveys nutrients from the periphery of the tubule to oocytes located deep within. The inner epithelium is composed of parietal and follicular epithelial cells and the oocytes. *Stichopus* oocytes contain three classes of microtubules based upon their location, orientation, and lability during fixation. Microtubules from the apical protuberance encircle the germinal vesicle. Cortical microtubules lie just under the cell surface and run parallel to it. Deep cytoplasmic microtubules run radially from the interior of the oocyte towards the cell surface. Oocytes are held within follicles by junctional complexes until the time of ovulation. Ovulation can be monitored in severed follicles of this species because an oolamina insures follicle integrity after detachment from the ovary. The onset of ovulation is marked by the dissolution of junctional complexes. This is followed by a cytochalasin B sensitive contraction of the follicle cells. The follicle contracts down around the oocyte, to lie collapsed against the ovarian wall while the oocyte is free within the ovarian lumen.

## INTRODUCTION

Ovulation in the aspidochirote holothurian *Stichopus californicus* (Stimpson, 1857) involves the extrusion of oocytes from epithelial follicles during normal spawning. Ovulation may be experimentally induced when excised ovaries or severed follicles are exposed to seawater. In the process of ovulation, junctions between oocytes and follicular epithelial cells break. This must occur before the oocytes are released and can subsequently mature. Ovulation has been described for asteroids (Schroeder, 1971; Schroeder *et al.*, 1979) and a crinoid (Holland and Dan, 1975), but little is known for holothurians. Study of the process is facilitated in this holothurian because ovulation will occur spontaneously in isolated ovarian fragments, and the ovulation of individual oocytes can be easily followed under the microscope. Analysis of holothurian ovulation must include a detailed understanding of the structure of the ovary and especially of the ovarian inner epithelium.

The anatomy and histology of the holothurian ovary is summarized in the major treatises of invertebrate zoology (Ludwig, 1889–1892; Cuenot, 1948; Hyman, 1955).

Received 3 June 1985; accepted 30 July 1985.

More detailed accounts of individual species appear in monographs (Gerould, 1896; Theel, 1901; Ohshima, 1921; Inaba, 1930; Menker, 1970). The holothurian ovary is an unpaired organ attached to the dorsal mesentery. It consists of a thick, fleshly central gonad basis and numerous blind ending tubules. The gonoduct exits the gonad basis from its dorsal anterior aspect and runs within the mesentery to the gonopore.

Holothurian oocytes are surrounded by a layer of squamous inner epithelial cells, called follicle cells. Ludwig (1889–1892) described a stalk supporting the oocytes in the aspidochirote holothurian *Holothuria mamorata,* but did not ascertain if the stalk was formed from follicle cells. Gerould's (1896) description of an intricate connection between the oocyte and follicle cells in the molpadiid holothurian *Caudina arenata* makes it clear that further investigation of these cells is required. Davis (1971) analyzed the gonad histology of a number of different echinoderm species using transmission electron microscopy. The focus of her study did not include a fine structural analysis of the inner epithelium of these species, although she did examine the structure of the ovarian wall of *Stichopus californicus.* There have been no electron microscopic analyses of the cellular relationships in the ovarian inner epithelium of a holothurian, nor are there reports of ovulation in holothurians or any experimental analysis of the process of ovulation.

This paper presents a detailed description of the fine structure of the ovarian fecund tubules in *Stichopus californicus,* and an experimental analysis of the process of ovulation. Our investigations corroborate the published descriptions of the general organization of the ovary and present new information on the cellular relationships within the inner epithelium of the fecund tubules. Our observation of three classes of microtubules within this oocyte may pertain to the expression of oocyte polarity. We extend previous descriptions of the fine structure of the investing peritoneum and connective tissue compartment, containing the genital hemal sinus. We also confirm reports of the complete resorption of the fecund ovarian tubules after spawning (Theel, 1901; Tyler and Gage, 1983). These results are discussed in light of the twin functions of the holothurian ovary, oogenesis and spawning, and compared with available descriptions of other echinoderms. The architectural simplicity and the primitive character of the holothurian ovary suggest that the fine structural descriptions presented here will be of value in understanding structure and function in other echinoderm ovaries.

## Materials and Methods

Specimens of *Stichopus californicus* were collected by dredging or by diving, and maintained in running seawater at The Friday Harbor Laboratories of the University of Washington. Ovaries were excised from healthy animals and fixed either in phosphate buffered glutaraldehyde (Cloney and Florey, 1968) or in a cacodylate buffered cocktail fixative (Eakin and Brandenburger, 1979). Osmication followed primary fixation and was done with the appropriate buffer. Isopropyl alcohol or acetone were used for dehydration. Propylene oxide was used as the antemedium before infiltration and embedment in Epon. One micron thick sections were stained with a mixture of azure II and methylene blue. Silver or silver-gold thin sections mounted on copper grids, stained with lead citrate and a solution of saturated uranyl acetate and methanol were viewed on a Philips EM 300 or 301 electron microscope. Glyoxilic acid staining for catecholamines was done by the method of Burke (1983).

*Stichopus* oocyte microtubules are not preserved when fixed in phosphate buffers. Microtubules of the protuberance are preserved with the cacodylate buffered cocktail fixative at room temperature and when the pH is 7.2. Preservation of cortical micro-

tubules is enhanced when the temperature of this fixative is kept below 4°C. The deep cytoplasmic microtubules are preserved with this fixation at 4°C and the pH of the buffer is between 7.5 and 7.8. We fixed portions of ovaries of more than 25 *Stichopus* individuals with each of these fixation regimes, then sectioned and examined them to determine the reliability of these fixations in preserving the different classes of oocyte microtubules. In all cases, microtubules are best preserved in oocytes that remain within the ovary.

Ovulation can be easily studied in *Stichopus californicus* because the entire process occurs spontaneously when the fecund tubules are torn open in sea water, and most oocytes ovulate quickly. Contraction of arrays of thin filaments has been implicated in a number of cellular movements (Cloney, 1966; Schroeder, 1972). Cytochalasin B disorganizes arrays of actin thin filaments and stalls their contraction (Schroeder, 1972, Wessels *et al.*, 1971). We used cytochalasin B prepared from a 1 mg/ml stock solution dissolved in dimethyl-sulfoxide (DMSO) in testing our hypothesis that actin filaments were involved in ovulation. The drug was diluted to 10 μg/ml with filtered seawater in the experiments. Controls containing 1% DMSO in filtered seawater had no effect on ovulation.

*Stichopus californicus* (Clark, 1922), hereafter called *Stichopus*, has also been referred to as *Parastichopus californicus* (Deichmann, 1937).

## Results

### *Anatomy of the gonad*

The ovary of stichopodid aspidochirote holothurians is a single bilaterally symmetrical organ; it consists of a gonad basis, numerous fecund tubules, and inconspicuous unripe tubules (Fig. 1). The gonad basis is a fleshy thickening in the dorsal suspensor mesentery to which all tubules attach. The tubules inserting on the flanks of the basis increase in size from anterior to posterior (Fig. 1). The largest, most posterior tubules are fecund before spawning and contain post-vitellogenic oocytes, 185–200 μm in diameter. In the late spring these tubules nearly fill the perivisceral coelom. The number of fecund tubules on the basis is variable, but there are usually between 10 and 12, and each branches dichotomously many times along its length. The aggregate lineal dimension of all the branches in a single tubule is about 38 cm. Fecund tubules average 3.1 mm in diameter but become thinner near their insertion on the basis. The volume of all the fecund tubules is about 185 ml during the late spring, close to the time of spawning.

### *Structure of the fecund tubules*

Fecund tubules of *Stichopus* have three layers, as determined by electron microscopy (Fig. 2). Outermost is a complex peritoneum continuous with the peritoneum that lines the perivisceral coelom. This complex peritoneum includes circular muscles and nerve fibers. A connective tissue compartment lies between the peritoneum and the inner epithelium. The connective tissue compartment contains the genital hemal sinus of the ovary. Longitudinal folds in the inner epithelium may extend several centimeters along the length of each tubule.

### *The outer layer of the ovary: the peritoneum*

*Epithelial cells.* The peritoneum is composed of squamous to low cuboidal epithelial cells that are joined by apical zonulae adherentes and subjacent septate junctions (Fig. 3). The apical surface of these epithelial cells characteristically bears a single

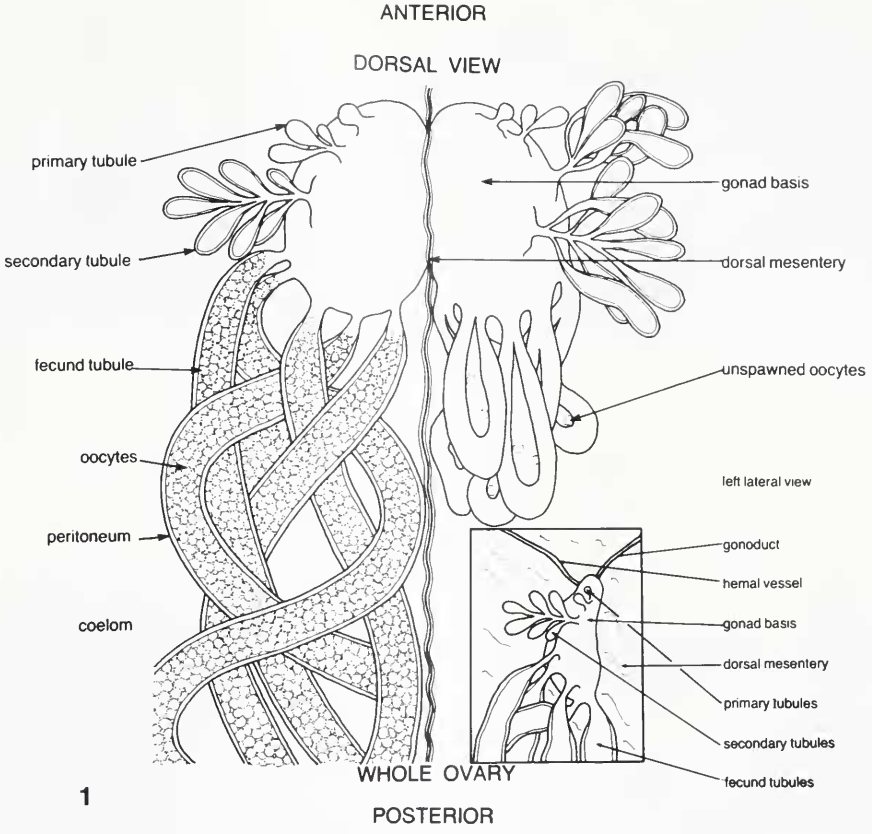



FIGURE 1. Whole ovary of *Stichopus californicus*. The left side represents the condition in the ovary prior to spawning, while the right side represents the post-spawning condition. The inset in the lower right depicts a left lateral view of the unspawned ovary.

cilium. Microvilli form a collar around the cilium and other microvilli cover the apical coelomic surface. Peritoneal epithelial cells contain dense arrays of rough endoplasmic reticulum that are uniformly distributed around the central nucleus. The epithelial cells often contain secondary lysosomes about 1.0 $\mu$m in diameter. The basal surface of the epithelial cells is associated with a basal lamina. Occasionally, hemidesmosomes are seen in muscle cells adjacent to the basal lamina (Fig. 4).

*Nerves.* Nerves are abundant in the ovarian peritoneum (Fig. 5). Each nerve is overlain by extensions of the peritoneal epithelial cells. Nerves are 2 to 4 $\mu$m in diameter and contain 25 to 250 axons. Assuming an average of 125 axons per nerve, 50 $\mu$m between nerves around the circumference of the tubule, and an average tubule circumference of 7 mm, the total number of axons in a single fecund tubule is approximately 24,000.

Axons are about 0.1 $\mu$m in diameter and contain microtubules, mitochondria, clear core vesicles about 50 nm in diameter, and dense core vesicles 80 to 150 nm in diameter. Some axons contain large vesicles 200 to 400 nm in diameter. Axon termini are enlarged to about 0.5 $\mu$m in diameter where the larger inclusions are found. Occasionally, aggregations of these large moderately staining vesicles are found in axons close to the hemal space (Fig. 5). No ovarian neuronal perikarya have been sectioned,

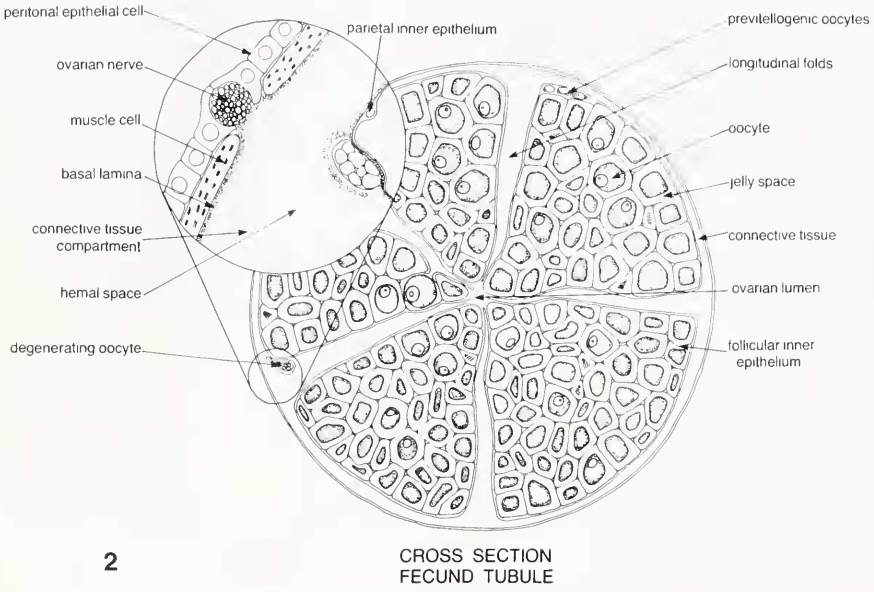



FIGURE 2. Cross-section of a fecund ovarian tubule illustrating the relative positions of the tissues. The inset circle represents a magnification of the smaller circle and depicts the organization of the complex peritoneum.

however, glyoxylic acid staining reveals that cell bodies containing reactive catecholamines are present in the outer layer of fecund tubules (Fig. 6). Neuromuscular junctions, membrane densitities, and gap junctions between axons and muscle cells have not been found. Because the nerve processes and the neuronal perikarya are superjacent to the peritoneal basal lamina we consider these nervous system elements part of this complex peritoneum.

*Muscle cells.* Light microscopy reveals a layer of muscle cells under the peritoneum in the ovarian tubules of *Stichopus.* However, electron microscopic examination of these muscle cells show they are also superjacent to the basal lamina of the peritoneum and lack an external lamina (Fig. 4). We infer that these muscle cells are myo-epithelial. The peritoneal muscle cells of the ovary form only a circular layer. Muscle cells occur every 20 $\mu$m along a tubule. A single tubule having an average length of 38 cm may, therefore, contain about 18,000 muscle cells.

Muscle cells nearly encircle the tubule, a distance of about 7 mm. Each appears to have a terminal perikaryon, but the position of the perikaryon is probably variable. Arrays of thick and thin filaments are similar to those in the smooth muscle of other echinoderms (Cavey and Wood, 1981). The diameter of thicker filaments is 50–65 nm, while that of the thinner filaments is 6–10 nm (Fig. 4). There is a reduced sarcoplasmic reticulum adjacent to the peritoneal basal lamina and mitochondria usually lie on the peritoneal side of the contractile elements. Desmosomes connect closely apposed muscle cells and dense plaques similar to hemidesmosomes occur on the basal surface of the cells. We found no gap junctions between muscle cells (Fig. 4).

### *Middle layer: the connective tissue compartment*

The connective tissue compartment of the ovary lies between the basal laminae of the peritoneum and the inner epithelium of the ovary. It contains ground substance,

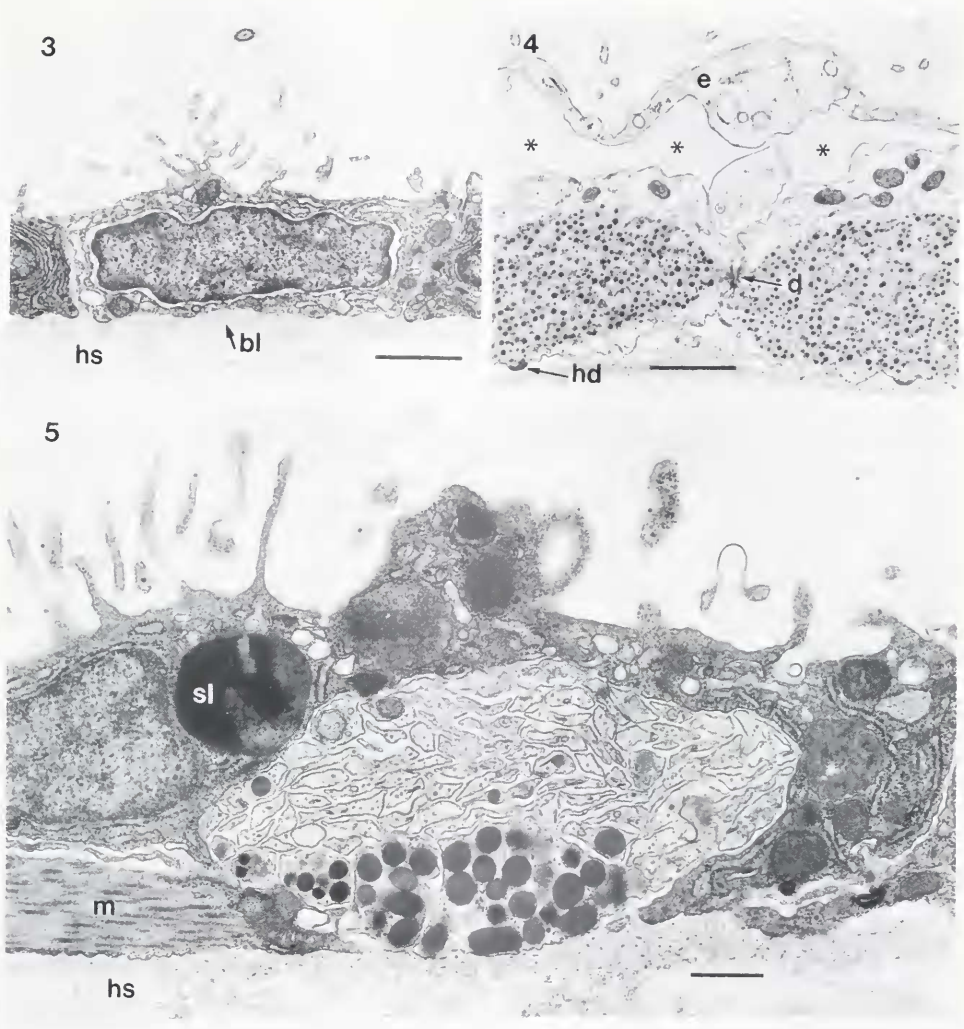



FIGURE 3. TEM of epithelial cells of the peritoneum. These cells lie on a basal lamina (bl) that separates them from the genital hemal sinus (hs) of the ovary. These cells often contain regular arrays of RER. Scale bar = 0.5 $\mu$m. 23,000×.

FIGURE 4. TEM of adjacent muscle cell contractile elements joined by a desmosome (d). The cells are attached to the basal lamina by hemidesmosomes (hd). Processes from epithelial cells (e) overlie the muscle cells. Spaces containing asterisks are fixation artifacts. Scale bar = 1.0 $\mu$m. 11,500×.

FIGURE 5. TEM of an ovarian nerve. Epithelial cells often bear secondary lysosomes (sl). Axons in this nerve lie on the basal lamina near the hemal space (hs) and contain large vesicles of moderate electron density. Muscle cells (m) underlie the epithelial cells and run perpendicular to the nerves. Scale bar = 0.5 $\mu$m. 19,500×.

fibers, fibroblasts, and the fluid filled sinus of the hemal system which carries some coelomocytes (Fig. 9).

Connective tissue fibers have the characteristics of collagen and the surrounding ground substance stains metachromatically with azure II. The fibers are about 35 nm in diameter and have a periodicity of about 65 nm (Fig. 8). The fibers are more densely aggregated near the basal lamina of the peritoneum than in other parts of the connective

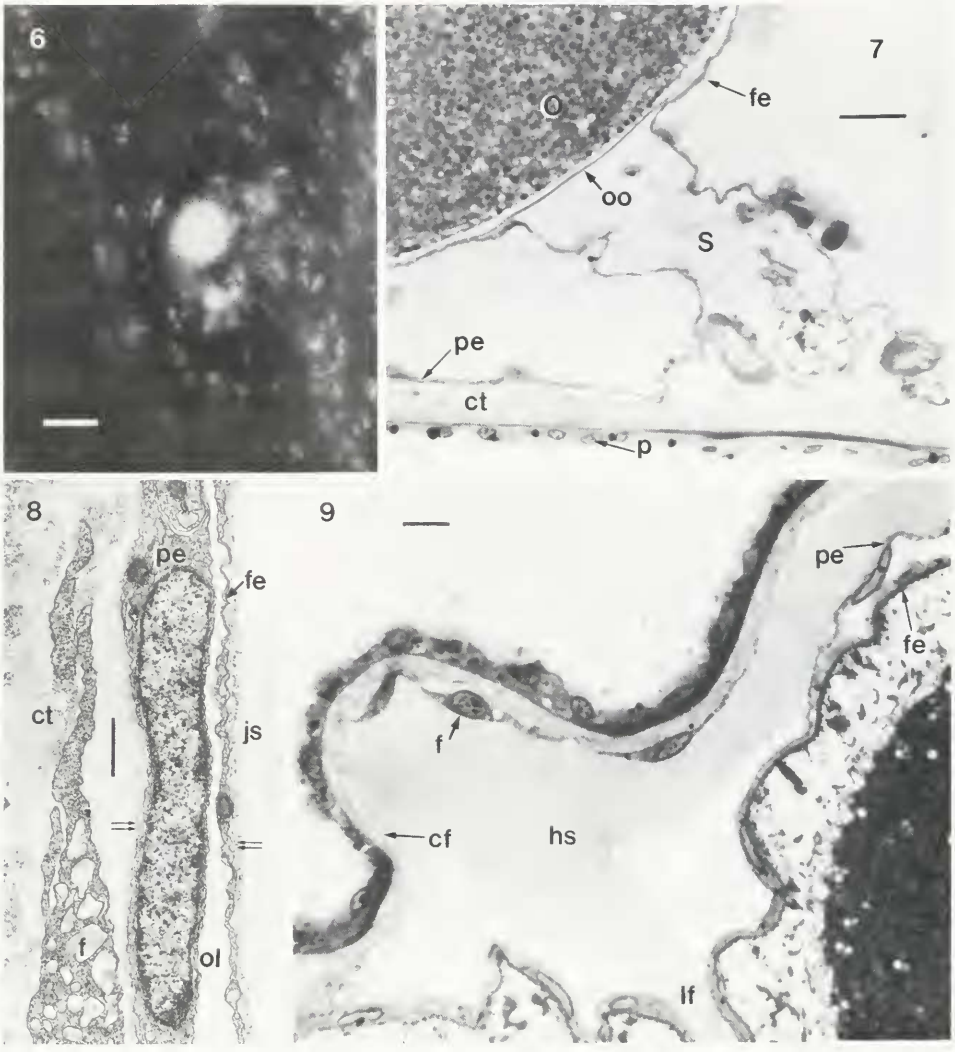



FIGURE 6. Glyoxylic acid induced catecholamine fluorescence of a putative neuronal perikaryon within the ovarian peritoneum. Scale bar = 10 $\mu$m. 710×.

FIGURE 7. Light micrograph (LM) of an oocyte (O) within its follicle. The follicular inner epithelium (fe) is continuous with the parietal inner epithelium (pe) at the stalk (S). The oocyte basal plate or oolamina (oo) separates the hemal sinus from the oocyte jelly space. The connective tissue compartment (ct), which includes the hemal sinus, spans the area between the peritoneal and inner epithelial basal laminae. Scale bar = 10 $\mu$m. 870×.

FIGURE 8. TEM of the closely applied apical surfaces of follicular (fe) and parietal (pe) inner epithelial cells. The basal laminae of these cells are indicated by double arrows. Within the connective tissues compartment (ct) is a fibroblast (f). The jelly space (js) surrounding the oocyte and the ovarian lumen (ol) are marked. Scale bar = 0.5 $\mu$m. 15,600×.

FIGURE 9. LM showing the organization of the connective tissue compartment with fibroblasts (f), fibers (cf), the serous hemal fluid within the hemal sinus (hs). The parietal (pe) and follicular (fe) inner epithelial cells are indicated, as well as the outermost part of a longitudinal fold (lf) in the inner epithelium. Scale bar = 10 $\mu$m. 620×.

tissue compartment. Fibroblasts usually occur among the aggregations of collagen fibers near the outer-most part of the connective tissue compartment of the fecund tubule (Fig. 9). Fibroblasts contain arrays of rough endoplasmic reticulum and bear long filopodial extensions, but their ultrastructure is otherwise unremarkable. Hemal fluid in the genital hemal sinus is a serous material that stains uniformly with Richardson's stain and appears flocculent in electron micrographs. Hemal fluid contains few cells; by far the most common are petaloid amoebocytes.

*Innermost layer: the ovarian inner epithelium*

The inner epithelium of the fecund ovarian tubules of *Stichopus californicus* is composed of three cell types; parietal inner epithelial cells, follicular inner epithelial cells, and oocytes. Parietal inner epithelial cells form the inner lining of the ovarian tubules and follicular inner epithelial cells make up the cellular follicles surrounding the oocytes (Figs. 7, 8, 9). These somatic cell types are continuous with one another at the follicular stalk and separate the ovarian connective tissue compartment from the ovarian lumen. The distinction between parietal and follicular inner epithelial cells, while largely one of convenience, serves to distinguish those inner epithelial cells which form a follicle around the oocyte and may have some potent endocrine function, as is the case in asteroids (Hirai and Kanatani, 1971).

*Somatic inner epithelial cells.* The somatic inner epithelial cells are squamous, often less than 0.5 $\mu$m. in thickness (Fig. 8). The cells are thicker near the nucleus and near the single apical cilium. In the thickened parts of the cells, mitochondria and substantial numbers of microtubules occur but there are few Golgi bodies and little rough endoplasmic reticulum. A collar of microvilli surrounds the cilium but no additional microvilli are found on the apical surface of the cells. These cells lie on a basal lamina; they are interdigitated and joined by zonulae adherentes, but never by septate junctions (Fig. 11). The interdigitating processes often contain numerous minute vesicles.

Follicular inner epithelial cells form the follicles surround the oocytes of the inner epithelium. The arrangement of cells within a folicle is depicted in Figure 10. At the point where the follicular and parietal inner epithelial cells abut, near the base of the follicular stalk, an especially thickened basal lamina, the oocyte basal plate or oolamina, underlies the oocyte (Figs. 7, 12). An unusual 'T' shaped connection between the oolamina and the basal laminae of the somatic cells is found at the nexus of parietal inner epithelial cells, the follicular inner epithelial cells and the oolamina. The oolamina is considerably thicker than somatic basal laminae although its ultrastructure is similar (Fig. 12). The oolamina forms a barrier between the genital hemal sinus and the subfollicular jelly space surrounding the oocyte, allowing only restricted association of the hemal fluid with the oocyte surface.

*Oocytes.* Oocytes of *Stichopus californicus* are 185–200 $\mu$m in diameter just before spawning (Fig. 29). The oocyte has a prominent germinal vesicle about 80 $\mu$m in diameter which usually contains one or two vesiculated nucleoli from which densely staining threads radiate. Oocytes from fecund tubules are postvitellogenic and contain numerous membrane bound yolk granules and other inclusions.

Holothurian oocytes bear an axial protuberance referred to in the literature as a "microplye appendage" (Ludwig, 1889; Gerould, 1896; Ohshima, 1921; Inaba, 1930). The *Stichopus californicus* oocyte protuberance inserts into the follicle cell capsule at a point roughly opposite the follicular stalk and basal plate (Fig. 13). A zonula adherens occurs between the protuberance and the follicle cells at the point of insertion (Fig. 15). The follicle cells contain numerous vesicles in this region, which are about 0.1 $\mu$m in diameter and have a clear core.

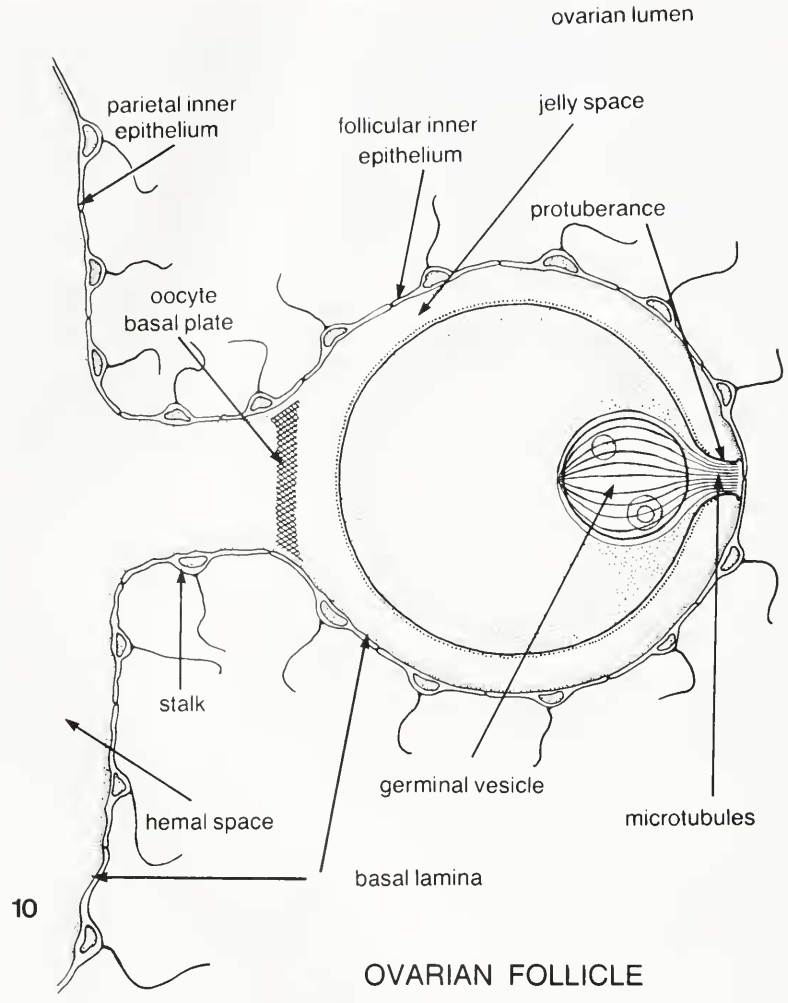



FIGURE 10. Relationships between the parietal and follicular inner epithelial cells and the oocyte. The oocyte basal plate or oolamina is the basal lamina of the oocyte.

The protuberance contains large numbers of microtubules which are more concentrated peripherally as determined by a comparison of medial and peripheral longitudinal sections (Figs. 14, 15). Polarization microscopy and TEM show that the microtubules of the protuberance extend into the ooplasm and surround the germinal vesicle (Fig. 17). These arrays of microtubules exclude granular inclusions from the protuberance and adjacent cytoplasm and produce striated zones in the oocyte near the animal pole (Fig. 13).

The oocyte cortex contains few bipartite granules reminiscent of those found in echinoids (Anderson, 1968). Differential interference contrast (DIC) microscopy of living oocytes reveals that the oocyte surface bears minute ridges or microplicae in addition to the microvilli (Fig. 21) which are best seen in Epon sections. Numerous microtubules are also found in the cortex under certain fixation regimes, but these are more labile than the microtubules of the protuberance (Fig. 16). Dense plaques

occur on the outer surface of the oocyte plasma membrane. A few vesicles close to the plasma membrane also have dense plaques as part of their inner surface, suggesting that the oocyte plasma membrane is in flux with the plasma membrane of these vesicles.

Deep within the oocyte cytoplasm another kind of microtubule is found (Fig. 18). These microtubules are extremely labile to fixation and are preserved consistently only when the pH of the fixation fluid is between 7.5 and 7.8 and the fixation done on ice (see Materials and Methods). These deep cytoplasmic microtubules run radially from the interior of the cell toward the periphery. They are oriented perpendicular to cortical microtubules and are not connected to the protuberance.

The oocytes contain abundant mitochondria which are evenly dispersed throughout the cytoplasm. Golgi bodies, putative pigment vesicles, and yolk granules are also uniformly distributed in the oocytes (Fig. 16). Some of the yolk granules contain crystalloid cores (Fig. 23). Unusual spherical annulate lamellae (Fig. 22) are found in the oocyte cytoplasm. The germinal vesicle is unremarkable other than the association of the protuberance microtubules with the nuclear envelope.

A jelly space separates oocytes from the basal lamina on the inner surface of the follicle capsule. Two components with distinct tinctorial properties occur in the jelly space of oocytes ligated prior to fixation. These may represent jelly coat precursors. Oocytes from torn tubules, which have been exposed to seawater, bear fully formed jelly coats regardless of whether they have ovulated. Ovulated oocytes lose their jelly coats during the processing for embedment.

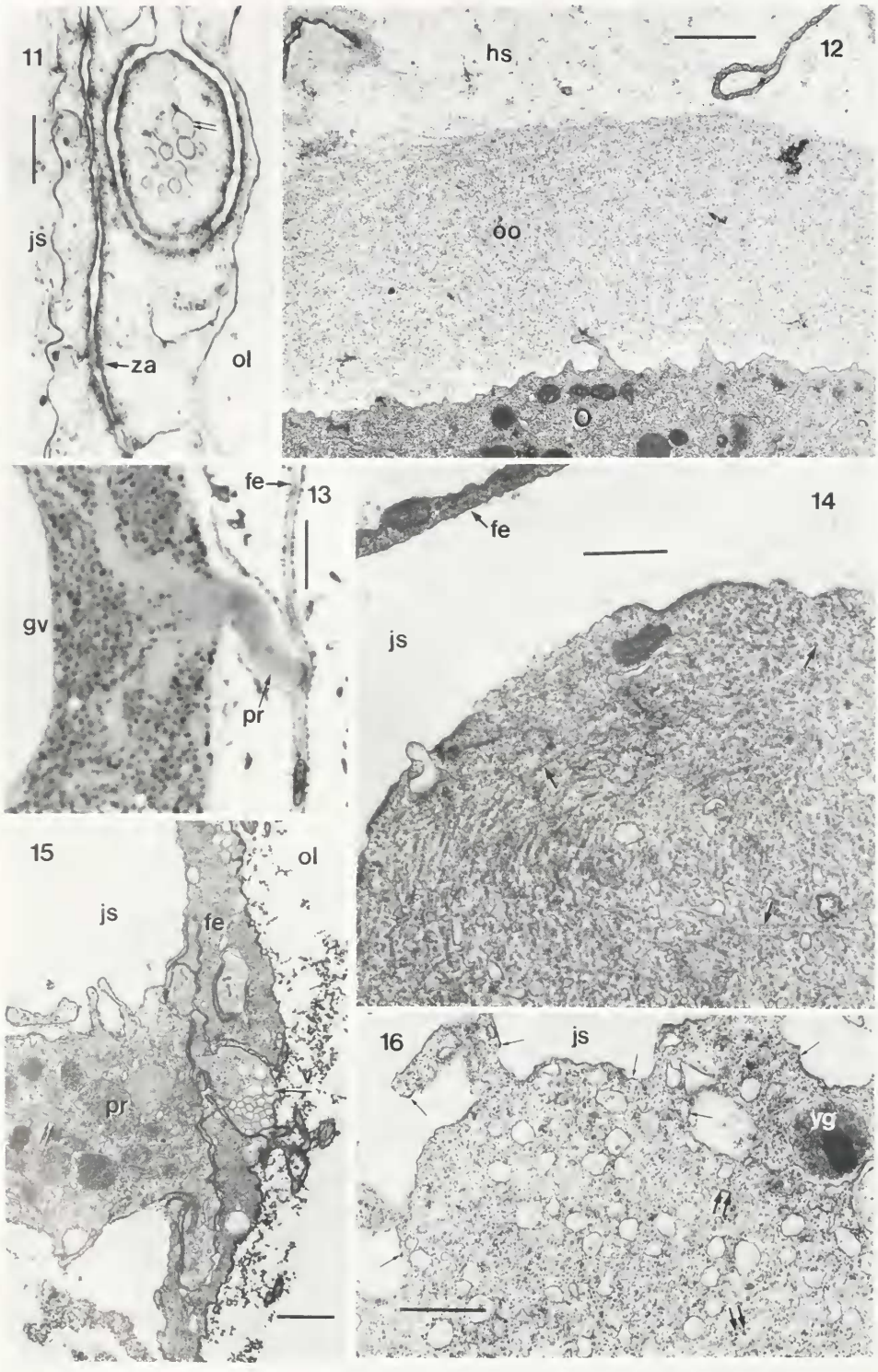
The ovarian lumen is restricted to the interstices between ovarian follicles in the fecund tubules; it sometimes contains petaloid amoebocytes (Fig. 20). The lumen of fecund tubules is directly connected to the gonoduct. Ovulated oocytes accumulate in the ovarian lumen until the time they are released from the gonoduct into the sea at spawning. Occasionally, both vesiculated and relict oocytes are found in the ovarian tubules (Figs. 19, 21). These are rare in freshly collected *Stichopus californicus* (Smiley, 1984).

### *Ovulation*

Ovulation can be defined as the severance of the intimate cellular connections between an oocyte and its surrounding ovarian somatic cells. The first event in ovulation in *Stichopus californicus* is the dissolution of the adhering junctions between the follicle cells and the protuberance of the oocyte. This is manifested by the movement of the protuberance through the follicle (Fig. 25). A small annulus in the follicle surrounds the protuberance at this time. The annulus enlarges and the follicle cells appear to retract down around the oocyte. The follicle cells are separated from the oocyte surface during this process by the jelly coat. As the annulus in the follicle enlarges, the oocyte begins to squeeze through the opening (Fig. 26). This process is accompanied by a distortion of the oocyte, causing it to assume an hourglass-like shape (Figs. 26, 27). The rate of follicle recession increases as ovulation continues, and the final stages are quickly completed (Figs. 27, 28). After ovulation, oocytes lie free in the lumen, and the follicle cells remain attached to the ovarian wall (Fig. 29).

Ovulation can be induced in intact ligated fecund tubules by treating ligated tubules with a solution of asteroid radial nerve extract (Smiley, 1984). Ovulation commences about three hours after the extract is added, and proceeds similarly to that of oocytes isolated in their follicles. Peristalsis of the tubule musculature continues for about four hours after the addition of the extract, and ends when the ovulated oocytes are forced through a severed end of the tubule by strong contractions of the musculature.

One hypothesis accounting for the forces required for ovulation is that hydration


11
js
za
ol
hs
12
oo
o
fe
13
gv
pr
14
fe
js
15
ol
js
fe
pr
16
js
yg

of the jelly coat provides hydrostatic forces that push the oocyte out of the follicle. This explanation is attractive because the jelly coat is first visible about the time of ovulation. To test this hypothesis directly, tears were made in the follicles of unovulated oocytes with fine glass needles, and ovulation was monitored. The data from 50 such experiments, performed on oocytes from 6 different ovaries, are presented in Table I. It is clear that a tear in the follicle does not stop ovulation.

Electron microscopic examination of the follicle cells during ovulation reveals thin filaments within these cells (Fig. 26). The diameter of the filaments is between 6 and 8 nm suggesting that they may be actin filaments. Schroeder (1971), on the basis of ultrastructural analysis of asteroid follicles, concluded that contraction of arrays of actin filaments within these follicles was responsible for providing some of the force that drives ovulation in starfish. To test the hypothesis that contraction of actin filaments within the follicle cells provides some of the force required for successful ovulation of *Stichopus* oocytes, pieces of torn fecund tubules were placed in filtered seawater containing the drug cytochalasin B. Because ovulation can occur immediately after the fecund tubules are torn open, scoring was based on the number of oocytes that were stalled in ovulation after incubation for 30 minutes in the drug solution. Therefore, oocytes completing ovulation before the drug could act were not counted. Table II presents data from these experiments as numbers of oocytes stalled in ovulation after 30 minutes. Results from the cytochalasin B treatment of ovulating oocytes supports the hypothesis that at least a part of the force required for ovulation is provided by contraction of cytochalasin B sensitive actin filaments.

### *Spawning and tubule resorption*

Ovulated oocytes are held within the tubule until the time of spawning. In uninduced spawns observed in aquaria, *Stichopus californicus* releases its oocytes as the germinal vesicles are breaking down. Based upon dissection of spawned animals from the field and the laboratory, spawning in *Stichopus californicus* is a catastrophic event which usually results in nearly complete evacuation of oocytes from fecund tubules. The release of oocytes considerably diminishes the size of the tubules, and virtually no normal oocytes remain in the tubules after spawning.

---

FIGURE 11. TEM of an interdigitation of follicle cells. Vesicles (double arrows) are found in the interdigitations. The cells are joined to one another with electron dense zonulae adherentes (za). The follicle cell basal lamina is next to the jelly space, and the ovarian lumen (ol) is on the opposite side. Scale bar = 0.25 μm. 39,200×.

FIGURE 12. TEM of the oocyte basal lamina, the oolamina (oo). This separates the oocyte (O) surface from the hemal sinus (hs) of the connective tissue compartment. Scale bar = 2 μm 5,700×.

FIGURE 13. LM of the oocyte protuberance (pr) in an oocyte attached to the follicle (fe). The striations within the protuberance appear to exclude yolk granules from areas at the apex of the oocyte. The large germinal vesicle nucleus (gv) is eccentric towards this apex. Scale bar = 10 μm. 1000×.

FIGURE 14. TEM of the protuberance in a grazing section showing the enormous number of microtubules (arrows) within the protuberance. The follicle cells (fe) are close by, and delimit the jelly space (js) surrounding the oocyte. Scale bar = 0.5 μm. 24,200×.

FIGURE 15. TEM of the insertion of the protuberance (pr) into the follicle cells (fe). The ovarian lumen (ol) and oocyte jelly space (js) are indicated. There are aggregations of vesicles within the follicle cells near the insertion of the protuberance (small arrow). Double arrows point out microtubules within the central region of the protuberance. The dense line between the protuberance and the follicle cells is the zonula adherens joining these cells. Scale bar = 1.0 μm. 8,200×.

FIGURE 16. TEM of the oocyte cortex. Dense plaques occur at the plasma membrane (single arrows) and within vesicles near the oocyte surface. Microtubules underlie the oocyte surface and are indicated by double arrows. A yolk granule (yg) near the oocyte surface contains a crystal-like regular array of filaments. Scale bar = 0.5 μm. 25,500×.

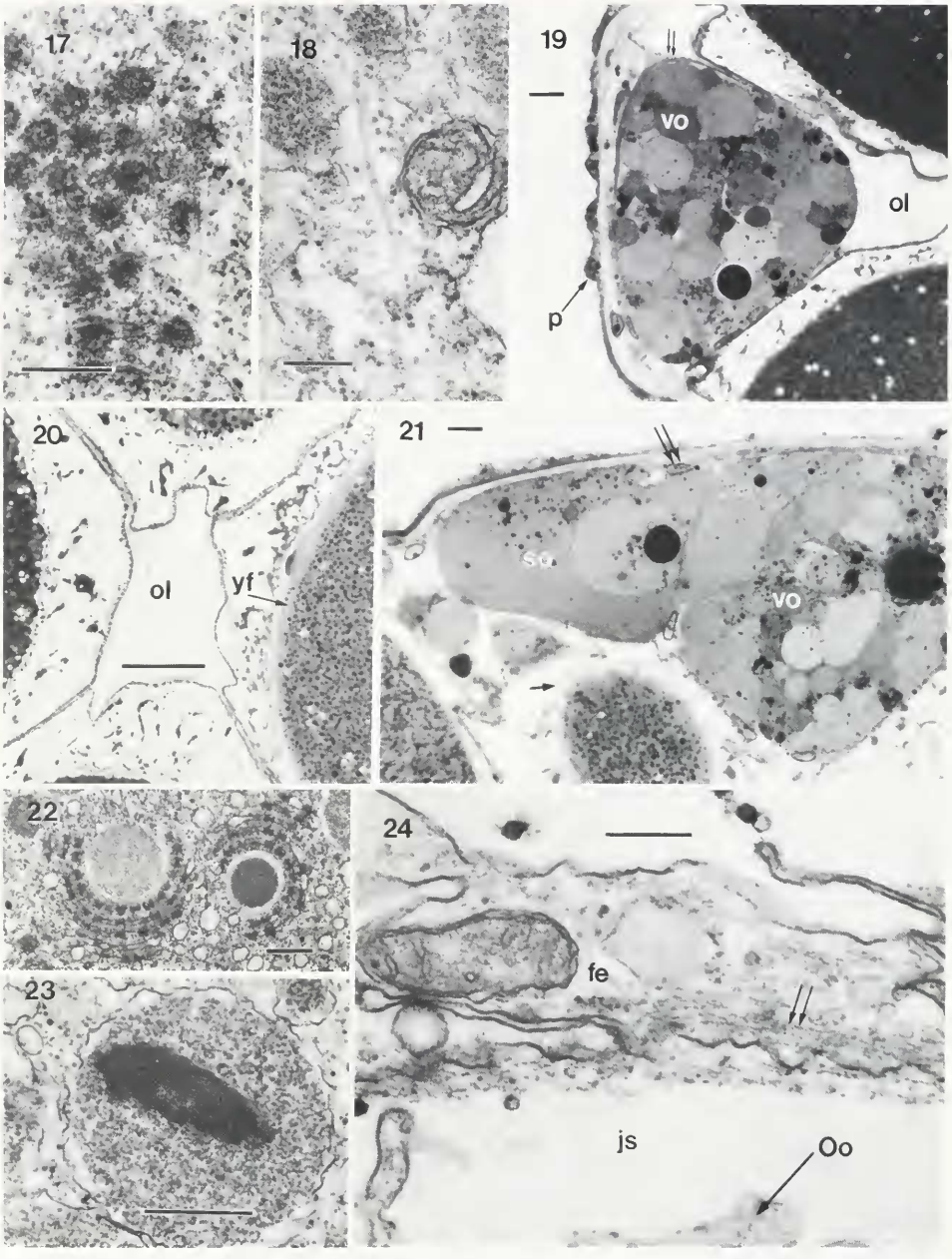



FIGURE 17. TEM of a grazing section of the nuclear envelope showing the nuclear pores and a microtubule. Microtubules appear to encircle the germinal vesicle. Scale bar = 0.25 $\mu$m. 48,000×.

FIGURE 18. TEM of a radially directed deep cytoplasmic mircotubule in the oocyte. The oocyte surface is about 20 $\mu$m beyond the top of the micrograph. Scale bar = 0.25 $\mu$m. 36,000×.

FIGURE 19. LM of a vesiculated oocyte (vo) in a fecund ovarian tubule. Vesiculated oocytes almost always occur at the periphery, near the peritoneum (p) of the ovary. They are separated from the ovarian lumen (ol) by cells of the inner epithelium. Double arrows indicate follicle cells. Scale bar = 10 $\mu$m. 420×.

Spawned tubules are resorbed within a few weeks. Resorption consists of further condensation in the length of the tubules and is accompanied by a visible deepening in their color. Sections through a resorbing tubule show the interior to be filled with partially degraded stunted oocytes and other cellular debris (data not shown). Marked cytolysis and phagocytosis of relict oocytes and other unidentifiable cells occurs. No follicle cells are found although there are numerous oolaminae visible. Secondary lysosomes and residual bodies abound in the perivisceral peritoneum of the resorbing tubules. Phagocytes containing dense aggregations of secondary lysosomes occur in the connective tissue compartment of these tubules and in some sections numerous morula cells also occur. Within several weeks, the only remnants of the fecund tubules are accumulations of dense pigment on the posterior of the gonad basis.

## Discussion

The ovary of *Stichopus californicus* and all other holothurians is an azygous organ located in the anterior dorsal coelom. In most, but not all holothurians, the gonad is bilaterally symmetic about the dorsal suspensor mesentery. The gonopore is located in interambulacrum CD. Among the other eleutherozoan echinoderms, the gonads are multiple, and each has interambulacral gonopores. The unit of structure of holothurian ovaries is the ovarian tubule. These are naked, not surrounded by an outer sac of tissue (Ludwig, 1889; Gerould, 1896; Theel, 1901; Ohshima, 1921; Inaba, 1930; Hyman, 1955; Menker, 1980; Tyler and Gage, 1983). In other eleutherozoan gonads, ascini are the units of structure and an outer sac is present (Davis, 1971; Atwood, 1973b; Schoenmakers *et al.*, 1981; Walker, 1982; Buckland-Nicks *et al.*, 1984). These differences in echinoderm ovarian structure may be resolved by comparing holothurian ovarian tubules not to the adult gonad of other eleutherozoan echinoderms, but to the genital rachis, which has a shape and histological composition similar to the holothurian gonad (Cuenot, 1948; Hyman, 1955).

### *Peritoneum*

In addition to serving as a protective layer for the ovary and containing the genital hemal sinus, the ovarian peritoneal epithelial cells probably absorb nutrients from the coelomic fluid. These cells bear numerous microvilli other than those which surround the single apical cilium. The fine structure is similar to the gonadal peritoneal epithelial cells of other holothurians (Atwood, 1973b; Davis, 1971). Krishnan and Dale (1975) suggest that the peritoneum of the testicular tubules of *Cucumaria frondosa* is capable of absorbing nutrients from the coelomic fluid. Nutrient absorption by peritoneal cells

---

FIGURE 20. LM showing the ovarian lumen (ol) in a fecund ovarian tubule. The ovarian lumen is reduced to these interstices between follicle bound oocytes within the ovary, and the oocyte is separated from its neighbor by two layers of follicle cells. The oocyte on the right has a yolk free zone (yf) at its cortex. Scale bar = 10 $\mu$m. 1100×.

FIGURE 21. LM of a stunted oocyte (so) at the periphery of a fecund tubule. Like vesiculated oocytes (vo), stunted oocytes are rarely found in the interior of the tubule, and are surrounded by inner epithelial cells (double arrow). A complex pattern of microvilli is visible on the surface of a normal post-vitellogenic oocyte cut in grazing section (single arrow). Scale bar = 10 $\mu$m. 450×.

FIGURE 22. TEM of unusual annulate lamellae within the oocytes. Scale bar = 0.5 $\mu$m. 12,500×.

FIGURE 23. TEM of a yolk vesicle within the oocyte. A crystalline like structure is present within the yolk. Scale bar = 0.5 $\mu$m. 29,100×.

FIGURE 24. TEM of follicle cell (fe) and oocyte surface (Oo) during ovulation. The jelly space (js) is indicated. Five filaments within the follicle cells are indicated by the double arrows. Scale bar = 0.25 $\mu$m. 46,000×.

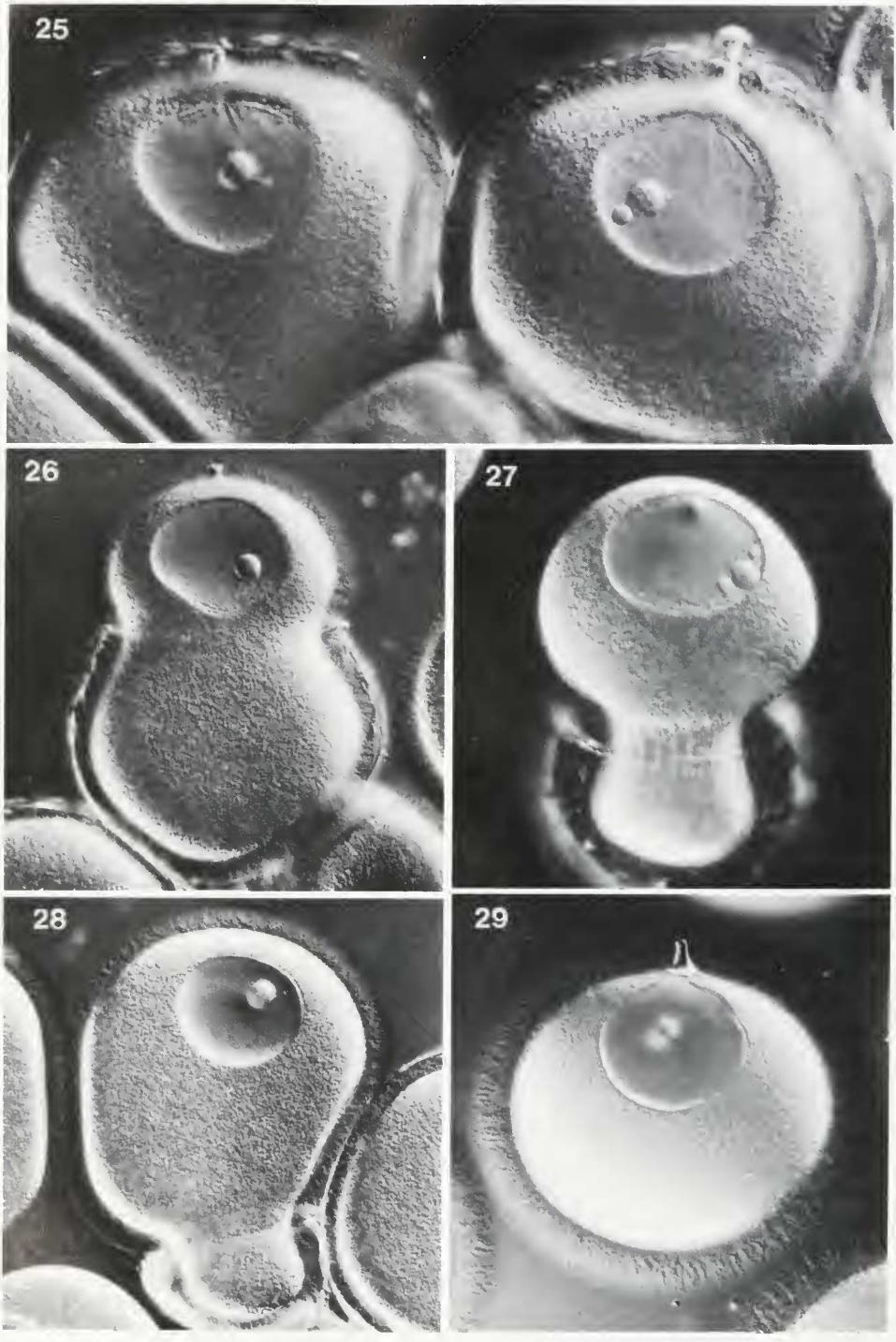



FIGURE 25. DIC micrograph of the first indication of ovulation. The protuberance (pr) of the oocyte on the left is still attached to its follicle cells. The protuberance of the oocyte on the right has poked through a small annulus in the follicle. 290×.

TABLE I

*Summary of results of operative experiments on ovulating oocytes in which each oocyte follicle was torn with fine glass needles*

| Total | Stalled | Ovulated | Dead |
|---|---|---|---|
| 50 | 4 | 39 | 7 |

along the length of the tubule coupled with the transfer of these nutrients to the genital hemal sinus could augment nutrient loads in the hemal fluid during vitellogenesis.

*Nerves.* Nerve processes are abundant in the peritoneum of the fecund ovarian tubules of *Stichopus californicus;* we estimate that as many as 24,000 axons may be present in each tubule. While the density of nerve processes is greater in the peritoneum of the *Stichopus californicus* ovarian tubules than has been reported for other eleutherozoan ovaries, it is difficult to estimate the total number of axons in those ovaries due to their complex shapes. This difficulty holds for perivisceral and hydrocoelic peritonea of other holothurians and other echinoderms (Atwood, 1973b; Baccetti and Rosati, 1968; Davis, 1971; Doyle, 1967; Jensen, 1975; Herreid *et al.,* 1976; 1977; Wood and Cavey, 1981). However, axons are plentiful in all examined echinoderm peritonea.

One hypothesis to explain the large number of axons in this ovarian peritoneum is derived by comparing the number of axons and muscle cells within a tubule. The correspondence of these numbers suggests that the mucle cells may be independently innervated. In all published reports of the fine structure of echinoderm perivisceral or hydrocoelic peritonea where dense assemblages of peritoneal myoepithelial cells are present, there are dense aggregations of axons. The possibility that muscle cells are independently innervated in this peritoneum is consistent with the absence of gap junctions between muscle fibers in this and a number of other echinoderm species (Prosser and Mackie, 1980; Cavey and Wood, 1981).

The aggregations of large, moderately electron dense vesicles (Fig. 5) are reminiscent of neurosecretory organs found in other animals (Baskin, 1976). Similarities in the size of the vesicles and their location suggests that they could have an endocrine function in this organ. The possibility that nerves within the echinoderm ovary could control oocyte maturation, ovulation, and spawning was suggested earlier for asteroids (Brusle, 1969; Holland, 1971; Atwood, 1973a). A potent neuroendocrine peptide is found in the radial nerves of a number of asteroids (Chaet and McConnaughy, 1959; Kanatani, 1979). Also, an aqueous solution of the radial nerve of the asteroid *Pycnopodia helianthoides* induces an increase in the percentage of oocytes of *Stichopus californicus* that undergo germinal vesicle breakdown (Strathmann and Satoh, 1969; Hufty and Schroeder, 1973). It is conceivable, therefore, that some endocrine factor may reside in the peritoneal neurons of this holothurian ovary.

FIGURE 26. DIC micrograph of the hour-glass stage of ovulation. The follicle lies about half way down the oocyte which is deformed at this point. 200×.

FIGURE 27. DIC micrograph of a late stage in ovulation. The follicle is particularly evident in this micrograph. The oocyte protuberance lies just out of focus over the germinal vesicle. 240×.

FIGURE 28. DIC micrograph of the final stage in ovulation. The oocyte has become nearly free of the follicle. This oocyte is slightly compressed. 180×.

FIGURE 29. DIC micrograph of an ovulated oocyte. The jelly coat surrounds the oocyte and the protuberance is topmost. 215×.

TABLE II

*Summary of results from experiments where torn tubules were placed in a solution of cytochalasin B. The numbers are an average of the number of oocytes stalled in ovulation after 30 minutes of exposure to 1% DMSO in filtered seawater (Control), or 10 μg/ml cytochalasin B in 1% DMSO filtered seawater (Experimental)*

| Six replicate experiments; different females. | |
|---|---|
| Control | Experimental |
| 2.17 | 47.5 |

*Peritoneal musculature.* The ovary of holothurians has been described as consisting of four layers, the peritoneum, a muscle layer, the connective tissue layer and the germinal epithelium (Ludwig, 1889; Gerould, 1896; Ohshima, 1921; Inaba, 1930). While the same layers appear in light micrographs presented in this study, detailed examination of the muscles in the *Stichopus californicus* ovary reveals that the muscle cells rest on the basal lamina of the peritoneum and have no external lamina. These facts support the interpretation that the muscle cells of this tissue are myoepithelial, and that the fecund ovarian tubule consists of only three principle layers. The same relationship of muscle cells to the peritoneal basal lamina has been found in other fine structural studies of perivisceral and hydrocoelic peritoneal epithelia in other holothurians (Atwood, 1973a; Baccetti and Rosati, 1968; Davis, 1971; Doyle, 1967; Doyle and McNiell, 1964; Herreid et al., 1976, 1977; Jensen, 1975; Pladellorens and Subirana, 1975). In the hydrocoelic or perivisceral peritonea of other echinoderms the musculature is superjacent to the peritoneal basal lamina and is interpreted as myoepithelial (Kawaguti, 1964, 1965; Cavey and Wood, 1981; Wood and Cavey, 1981). Further work is needed to determine if the peritoneal musculature of echinoderms is derived from the epithelial cells of the peritoneum or from mesenchyme.

In the gonads of some holothurians, longitudinal as well as circular muscles are present (Hyman, 1955; Davis, 1971; Atwood, 1973b; Franklin, 1980), while in others, only circular muscles are found (Gerould, 1896). The muscle layer of the fecund tubules of *Stichopus californicus* has a circular but no longitudinal component. A coordinated contraction of these circular muscles beginning at the distal end of the tubule and proceeding proximally is probably required to completely evaluate the tubules during spawning. Those holothurians that release only a portion of their gametes at any single spawn during a reproductive season may have more elaborate control over their ovarian musculature. *Stichopus californicus* releases its eggs in a slow steady stream and not with the propulsive force reported for some tropical holothurians (Mosher, 1982). The differences in the force of gamete expulsion probably reflect differences in the organization of the tubule musculature as well as adaptations to particular ecological conditions.

In addition to its function in spawning, the peritoneal musculature of the tubule functions in peristalsis, which is visible in the tubules when the body cavity is opened. We suggest that peristalsis could mix nutrient rich hemal fluid at the periphery of the tubule with nutrient depleted hemal fluid deep in the longitudinal folds. Peristaltic contractions are more local and weaker than induced spawning contractions. These differences may reflect contractions of different sets of muscle cells or they may reflect the use of distinct neurotransmitters for each event. The presence of two kinds of vesicles within the peritoneal neurons, clear core and dense core, is compatible with both these suggestions.

*Connective tissue compartment*

The genital hemal sinus is the most obvious component of the ovarian connective tissue compartment in *Stichopus*. The sinus is a channel without cellular boundaries, with an organization similar to that of other echinoderms (Ruppert and Carle, 1984). A possible exception is the hemal 'vessel' of the holothurian *Cucumaria frondosa* (Doyle and McNiell, 1964) which is reported to bear an incomplete cellular inner lining or endothelium of fibroblast-like cells.

The histology of the genital hemal sinus within the ovary lends credence to the supposition that it supplies nutrients to the developing oocytes (Ludwig, 1889; Hyman, 1955; Walker, 1982; Ferguson, 1984). The role of the genital hemal sinus in providing these nutrients would be clarified if two questions were answered. First, how is longitudinal translocation of the nutrients along the length of the tubule possible? Second, how is radial translocation or replenishment of nutrient concentrations deep within the ovary accomplished?

On the basis of the hydrodynamic problems involved, it is difficult to support the contention that the longitudinal translocation of hemal fluid from the dorsal hemal sinus to the tips of the fecund tubules is the major route of nutrient transfer in the ovary. However, if nutrients are taken up by the peritoneum and passed to the hemal sinus all along the length of the tubule, it is possible to explain the radial translocation of the nutrient load toward the more centrally located oocytes by peristaltic contraction of the peritoneal musculature.

*Inner epithelium*

The inner epithelium of the *Stichopus californicus* ovary is continuous throughout the tubules, the gonad basis, and the gonoduct. The inner epithelium of fecund tubules consists of somatic and germ cells. The somatic cells are squamous for the greater part of the tubule's length, but close to the point where the tubule inserts into the gonad basis, the somatic cells become more cuboidal and the germ cells are absent.

Our description of the inner epithelium of the ovary supports previous observations on other holothurians (Ludwig, 1889; Gerould, 1896; Ohshima, 1921; Inaba, 1930). We have demonstrated that the oocytes are held within their follicles by intercellular junctions until the time of ovulation. While it is not certain that a cellular follicle surrounds all echinoderm oocytes, cellular follicles surround oocytes of ophiuroids (Patent, 1968) and asteroids (Schroeder *et al.*, 1979; Schoenmakers *et al.*, 1981). Strands of tissue, that may be cellular, surrounding echinoid oocytes have been depicted in drawings (Tennent and Ito, 1941) and micrographs (Pearse, 1970). In crinoids, oocytes are reflected into a basal lamina-lined cavity in the genital hemal sinus, but oocytes ovulate through a somatic inner epithelium (Holland, 1971; Holland and Dan, 1975). Although the primary function of somatic inner epithelial cells in all echinoderms is probably protection and the creation of a physiologically controllable microenvironment around the oocyte, some asteroid follicles act to produce the oocyte maturation hormone 1-methyladenine (Hirai and Kanatani, 1971). No endocrine function has been specifically localized in holothurian follicle cells.

*Oocyte*

*Protuberance.* The holothurian oocyte protuberance has been called the "micropyle appendage" to indicate homology between this structure and the jelly canal (micropyle) of the sea urchin ovum (Gerould, 1896; Ohshima, 1921). This term is confusing because it suggests that the oocyte is fertilizable at only one point on its surface. This suggestion

is contradicted by our observations and is not corroborated in primary sources. It is likely that the recent report that sperm entry can occur at only one point on the holothurian oocyte surface is based on a misinterpretation of this term (Nieuwkoop and Sutasurya, 1983).

The oocyte protuberance has several functions; first it is the site of attachment of the oocyte to the follicle cells, second, its appearance is temporally associated with the migration of the germinal vesicle to its eccentric position in this holothurian, and third, it is the site of polar body formation, and marks the animal pole of the oocyte (Maruyama, 1980; Smiley, 1984).

Asteroid oocytes appear to be held within their follicle by basally directed processes from the follicle cells that make stable contacts with the oocyte surface (Schroeder *et al.,* 1979; Schroeder, 1981). It is likely that these processes serve the same attachment function in asteroids as the protuberance does in *Stichopus.* Information on how oocytes are attached to the inner epithelium is incomplete for both ophiuroids and echinoids. In the crinoid *Comanthus japonica,* the oocyte appears to have a structure somewhat similar to the holothurian protuberance (Fig. 11, Holland *et al.,* 1975).

The cytoplasmic striations associated with the holothurian oocyte protuberance have been reported to be homologous with a structure in asteroid oocytes called the polar plate (Buchner, 1911; Lindahl, 1932). The asteroid polar plate as well as similar axial structures found in oocytes of echinoids (Monne, 1946) and crinoids (Holland *et al.,* 1975) may serve as a center of aggregation of microtubules as does the protuberance of *Stichopus californicus.* This hypothesis is supported by the discovery of an ordered aggregation of microtubules between the germinal vesicle and the presumptive animal pole in the oocytes of the asteroid *Pisaster ochroceus* (Otto and Schroeder, 1984), although these investigators refer to this array of microtubules as a "premeiotic aster". The homology of this structure with the holothurian oocyte protuberance is supported on the basis of its location as well as its microtubular composition. The microtubules of the *Stichopus californicus* protuberance can be distinguished from the cortical microtubules in *Stichopus* and in the asteroid *Pisaster ochraceus* (Otto and Schroeder, 1984; Schroeder and Otto, 1984), by their axial location, their orientation, and their association with the nuclear envelope of the germinal vescile.

The protuberance develops late during oogenesis in *Stichopus,* when the oocyte has reached a diameter greater than 150 $\mu$m (Smiley, 1984). Late development of the protuberance also occurs in several other holothurians (Ohshima, 1921; Inaba, 1930), but in *Caudina arenata,* it is present in very small oocytes (Gerould, 1896). In all holothurian oocytes described, the germinal vesicle is centrally located until the time the protuberance becomes visible. Movement of the germinal vesicle to an eccentric position during vitellogenesis has also been reported in the holothurians *Cucumaria echinata* and *Caudina chilensis* (Ohshima, 1921; Inaba, 1930) and in the asteroid *Leptasterias hexactis* (Chia, 1968). The association of microtubules with the germinal vesicle in *Stichopus,* and the temporal concurrence of the development of the protuberance and the establishment of germinal vesicle eccentricity, suggests that the microtubules found within the protuberance may be involved in the movement of the germinal vesicle to its eccentric position, or its anchorage there. Otto and Schroeder (1984) suggested a similar function for the homologous microtubular aggregations in *Pisaster ochraceus.* Because germinal vesicle migration occurs at about the time of germinal vesicle breakdown in oocytes of *Holothuria leucospilota* and *Holothuria pardalis,* which are treated with dithiothreitol to induce oocyte maturation (Maruyama, 1980), the control of germinal vesicle migration may not be universal among holothurians.

The importance of three separable classes of microtubules within the *Stichopus* oocyte is not resolved. We have interpreted the microtubules of the protuberance as distinct from those of the cortex, as do Otto and Schroeder (1984) for the asteroid *Pisaster ochraceus*. On the basis of their differential stability in fixation, their distinct location, and different orientation, we also interpret the deep cytoplasmic microtubules found in *Stichopus* oocytes as a separate class of microtubules. This class of deep cytoplasmic microtubules has not, to our knowledge, been reported for any other oocyte. The difficulty in preserving these microtubules suggests that they may not have been preserved in other oocytes, although they could be present in the living cell.

The holothurian oocyte protuberance is a much more visible marker of oocyte polarity than the pigment band of *Paracentrotus lividus* oocytes (Boveri, 1901). The fact that the protuberance marks the site of polar body formation indicates the potential usefulness of this polarized axial marker in experimental manipulations of oocytes directed toward determining the cytological and biochemical properties of oocyte polarization.

### *Oolamina*

Oocytes abut the hemal sinus at the oolamina, or oocyte basal plate. Because of its size and metachromatic staining, the oolamina is a convenient marker for the vegetal pole of the oocyte and for following changes in the ovary after ovulation. Nutrients in the hemal sinus must cross this barrier to enter the jelly space and contact the oocyte surface. In addition to acting as a sieve for the materials in the genital hemal sinus fluid, the oolamina may restrict passage of materials out of the jelly space, such as the maturation hormone during the hormone dependent period (Kanatani, 1979). Our ovulation studies also show that the oolamina can insure the integrity of the follicle if the stalk is severed from its connection with the parietal inner epithelium.

### *Ovulation*

The junctions between oocytes and somatic inner epithelial cells in echinoderm ovaries must be ruptured before oocytes can be released into the sea. This may explain why asteroids and holothurians do not release oocytes when injected with isotonic potassium chloride solutions (Hyman, 1955). Ovulation has been described for a crinoid (Holland and Dan, 1975) and several asteroids (Schroeder, 1971). Crinoid ovulation involves distortion of the oocyte into an hourglass shape but contraction of the somatic cells of the inner epithelium has not been reported. In asteroid ovulation, follicle cells contract more independently and oocyte distortion is minimal. During ovulation in *Stichopus californicus,* the oolamina and follicle cells remain attached to the inner epithelium of the ovary, and ovulated oocytes accumulate in the ovarian lumen. With the collapse of the oocyte follicles, the ovarian lumen encompasses the greater part of the volume of the ovary. Continuous and asynchronous peristaltic contractions of the peritoneal musculature begin at the distal end of the ovarian tubule and move the oocytes within the lumen. Later, oocytes will spill from the gonoduct of the animal. The jelly coats of spawned oocytes are intact; in *Stichopus,* oocytes are not spawned with their follicles, though the release of oocytes in their follicles has been reported for *Cucumaria elongata* (Chia and Buchanan, 1968).

The experimental analysis of the process of ovulation does not prove that contraction of actin filaments produces all the forces involved in ovulation, but it does indicate that actin filament contractions are involved. It is unfortunate that the oocytes

of *Stichopus californicus* are intensely autofluorescent at the wavelength of the fluorophores fluorescein isothiocyanate and NDB. When different fluorophores are available, it would be interesting to study the involvement of microfilaments in the oocytes as well as in the follicle cells to determine the relative contribution of each of the process of ovulation.

*Resorption of spawned tubules*

*Stichopus californicus* completely resorbs the spawned fecund tubules in the weeks immediately following spawning. The process of resorption in *Stichopus* is very similar to that reported for *Mesothuria intestinalis* (Theel, 1901) and *Ypsilothuria talismani* (Tyler and Gage, 1983). Post spawning resorption of spent tubules was reported for other holothurians by Hyman (1955) who suggested that it would prove to be the rule among holothurians. Investigations on the reproductive biology of several holothurians do not indicate that resorption has occurred after spawning (Tanaka, 1958; Conand, 1981). The complete resorption of the fecund tubules in *Stichopus* obscures the source of future oocytes, unless they come from the smaller tubules more anterior on the gonad basis.

## ACKNOWLEDGMENTS

We thank the administrators and staff of The Friday Harbor Labs for the use of the facilities, their encouragement and help. The cytochalasin B was a gift from Dr. T. E. Schroeder. We appreciate the advice of many faculty and students at Friday Harbor and the Zoology Department, University of Washington. Particularly helpful were: G. Freeman, M. Hille, K. Irons, and W. Moody. S.S. is supported by NIH training grant HD-00266.

## LITERATURE CITED

ANDERSON, E. 1968. Ooctye differentiation in the sea-urchin *Arbacia punctulata*, with special reference to the origin of cortical granules and their participation in the cortical reaction. *J. Cell Biol.* **37:** 514–539.

ATWOOD, D. G. 1973a. Correlation of gamete shedding substance with the presence of neurosecretory granules in asteroids. *Gen. Comp. Endocrinol.* **20:** 347–350.

ATWOOD, D. G. 1973b. Ultrastructure of the gonad wall of the sea cucumber *Leptosynapta clarki*. *Z. Zellforsch.* **141:** 319–330.

BACCETTI, B., AND F. ROSATI. 1968. The fine structure of the polian vesicles of holothurians. *Z. Zellforsch.* **90:** 148–160.

BASKIN, D. G. 1976. Neurosecretion and the endocrinology of nereid polychaetes. *Am. Zool.* **16:** 107–124.

BOVERI, T. 1901. Die Polaritat von Ovocyte, Ei, und Larve des *Strongylocentrotus lividus*. *Zool. Jahrb.* **14:** 630–653.

BRUSLE, J. 1969. Aspects ultrastructuraux de l'innervation des gonades chez l'etiole de mer *Asterina gibbosa*. *Z. Zellforsch.* **98:** 88–98.

BUCHNER, P. 1911. Die Reifund des Seesternes bei experimenteller Parthenogenese. *Arch. Zellforsch.* **6:** 577–612.

BUCKLAND-NICKS, J., C. W. WALKER, AND F. S. CHIA. 1984. Ultrastructure of the male reproductive system and of spermatogenesis in the viviparous brittle star, *Amphipholis squamata*. *J. Morphol.* **179:** 243–262.

BURKE, R. D. 1983. The development of the larval nervous system of the sand dollar *Dendraster excentricus*. *Cell Tissue Res.* **218:** 475–485.

CAVEY, M. J., AND R. L. WOOD. 1981. Specializations for excitation contraction coupling in the podial retractor cells of the starfish *Stylasterias forreri*. *Cell Tissue Res.* **218:** 475–485.

CHAET, A. B., AND R. A. MCCONNAUGHY. 1959. Physiologic activity of nerve extracts. *Biol. Bull.* **117:** 407.

CHIA, F. S. 1968. Some observations on the development and cyclic changes of the oocytes in a brooding starfish. *J. Zool.* **154:** 453–461.

CHIA, F. S., AND J. B. BUCHANAN. 1969. Larval development of *Cucumuria elongata*. *J. Mar. Biol. Assoc. U. K.* **49**: 151–159.

CLARK, H. L. 1922. The holothurians of the genus *Stichopus*. *Bull. Mus. Comp. Zool.* **65**: 39–74.

CLONEY, R. A. 1966. Cytoplasmic filaments and cell movements: epidermal cells during ascidian metamorphosis. *J. Ultrastruct. Res.* **14**: 300–328.

CLONEY, R. A., AND E. FLOREY. 1968. The ultrastructure of cephalopod chromatophore organs. *Z. Zellforsch.* **89**: 250–280.

CONAND, C. 1981. Sexual cycle of three commercially important holothurian species from the lagoon of New Caledonia. *Bull. Mar. Sci.* **31**: 523–543.

CUENOT, L. 1948. Anatomie, ethologie, et systematique des echinodermes. In *Traite de Zoologie*, P. Grasse, ed. **11**: 3–275. Masson & Cie, Paris.

DAVIS, H. S. 1971. The gonad walls of the Echinodermata: a comparative study based on electron microscopy. M.Sc. Thesis U. C. San Diego. 90 pp.

DEICHMANN, E. 1937. The Templeton Crocker Expedition 9. Holothurians from the Gulf of California, the west coast of Lower California, and Clarion Island. *Zoologica* **22**: 161–176.

DOYLE, W. L. 1967. Vesiculated axons in the hemal vessel of a holothurian *Cucumaria frondosa*. *Biol. Bull.* **132**: 329–336.

DOYLE, W. L., AND G. F. MCNIELL. 1964. The fine structure of the respiratory tree in *Cucumaria frondosa*. *Q. J. Microsc. Sci.* **105**: 7–11.

EAKIN, R. M., AND J. L. BRANDENBURGER. 1979. Effects of light on ocelli of seastars. *Zoomorphologie* **92**: 191–200.

FERGUSON, J. C. 1984. Translocative functions of the enigmatic organs of starfish—The axial organ, hemal vessels, Tiedemann's bodies, and rectal caeca: an autoradiographic study. *Biol. Bull.* **166**: 140–155.

FRANKLIN, S. E. 1980. The reproductive biology and some aspects of the population ecology of the holothurians *Holothuria leucospilota* and *Stichopus chloronotus*. PhD. Diss. Univ. Sydney, Australia.

GEROULD, J. H. 1896. Anatomy and histology of *Caudina arenata*. *Bull. Mus. Comp. Zool.* **29**: 124–190.

HERREID, C. F., V. F. LARUSSA, AND C. R. DEFESI. 1976. Blood vascular system of the sea cucumber *Stichopus moebii*. *J. Morphol.* **150**: 423–452.

HERREID, C. F., V. F. LARUSSA, AND C. R. DEFESI. 1977. Vascular follicle system of the sea cucumber *Stichopus moebii*. *J. Morphol.* **154**: 19–38.

HETZEL, H. R. 1960. Studies on the coelomocytes of the sea cucumber *Cucumaria miniata*. PhD. Diss. Univ. Washington. 201 pp.

HIRAI, S., AND H. KANATANI. 1971. Site of production of meiosis-inducing substance in the ovary of starfish. *Exp. Cell Res.* **67**: 224–227.

HOLLAND, N. D. 1971. The fine structure of the ovary of a feather star, *Nemaster rubiginosa*. *Tissue Cell* **3**: 163–177.

HOLLAND, N. D. 1981. Electron microscopy of development in a sea cucumber, *Stichopus tremulus*, from unfertilized egg through hatched blastula. *Acta Zool.* **62**: 89–112.

HOLLAND, N. D., AND K. DAN. 1975. Ovulation in an echinoderm. *Experientia* **31**: 1078–1079.

HOLLAND, N. D., J. C. GRIMMER, AND H. KUBOTA. 1975. Gonadal development during the annual reproductive cycle of *Comanthus japonica*. *Biol. Bull.* **148**: 219–242.

HUFTY, H. M., AND P. C. SCHROEDER. 1974. A hormonally active substance produced by the ovary of the holothurian *Parastichopus californicus*. *Gen. Comp. Endocrinol.* **23**: 348–351.

HYMAN, L. H. 1955. *Echinoderms*, Vol. IV, *The Invertebrates*. McGraw-Hill. New York. 763 pp.

INABA, D. 1930. Notes on the development of a holothurian, *Caudina chilensis*. *Sci. Rept. Tohoku Univ. Ser. 4 Biol.* **5**: 215–248.

JENSEN, H. 1975. Ultrastructure of the dorsal hemal vessel in *Parastichopus*. *Cell Tissue Res.* **160**: 335–369.

KANATANI, H. 1979. Hormones in echinoderms. Pp. 273–307 in *Hormones in Evolution Vol I*, E. J. Barrington, ed. Academic Press, NY.

KAWAGUTI, S. 1964. Electron microscopy of the intestinal wall of the sea cucumber with special attention to its muscle and nerve plexus. *Biol. J. Okayama Univ.* **10**: 39–50.

KAWAGUTI, S. 1965. Electron microscopy of the ovarian wall of the echinoid with special reference to its muscle and nerve plexus. *Biol. J. Okayama Univ.* **11**: 66–74.

KRISHNAN, S., AND T. DALE. 1975. Ultrastructural studies on the testes of *Cucumaria frondosa*. *Norw. J. Zool.* **23**: 1–15.

LINDAHL, P. E. 1932. Zur Kenntnis des Ovarialeies Bei dem Seeigel. *Wilhelm Roux's Arch.* **126**: 373–390.

LUDWIG, H. 1889-1892. Bronn's *Klassen und Ordnungen des Thier Reiches*. Band 2, Buch I. *Die Seewaltzen*. C. F. Winter'sche Verlagshandlung Leipzig. 460 pp.

MARUYAMA, Y. K. 1980. Artificial induction of oocyte maturation and development in the sea cucumbers *Holothuria leucospilota* and *Holothuria pardalis*. *Biol. Bull.* **158**: 339–348.

MENKER, D. 1970. Lebenszyklus, jugendentwicklung und Geschlechtorgane von *Rhabdomolgus ruber. Mar. Biol.* **6:** 167–186.

MONNE, L. 1946. Some observations on the polar and dorsoventral organization of the sea urchin egg. *Ark. f. Zool.* **38A:** No. 15: 1–13.

MOSHER, C. 1982. Spawning behavior of the aspidochirote holothurian *Holothuria mexicana.* Pp. 467–468 in *Echinoderms: Proceedings of the International Conference, Tampa Bay,* J. M. Lawrence, ed. Balkema Press, Rotterdam.

NIEUWKOOP, P. A., AND L. A. SUTASURYA. 1981. *Primordial Germ Cells of the Invertebrates.* Cambridge Univ. Press, Cambridge. 258 pp.

OHSHIMA, H. 1921. On the development of *Cucumaria echinata. Q. J. Microsc. Sci.* **65:** 173–246.

OTTO, J. J., AND T. E. SCHROEDER. 1984. Microtubule arrays in the cortex and near the germinal vesicle of immature starfish oocytes. *Dev. Biol.* **101:** 274–281.

PATENT, D. 1968. The general and reproductive biology of the basket star *Gorgonocephalus caryi.* PhD. Diss. U. C. Berkeley. 128 pp.

PEARSE, J. S. 1970. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez III. The echinoid *Diadema setosum. Bull. Mar. Sci.* **20:** 697–720.

PLADELLORENS, M., AND J. A. SUBIRANA. 1975. Spermiogenesis in the sea cucumber *Holothuria tubulosa. J. Ultrastruct. Res.* **52:** 235–242.

PROSSER, C. L., AND MACKIE, G. O. 1980. Contractions of holothurian muscle. *J. Comp. Physiol.* **136:** 103–112.

RUPPERT, E. E., AND K. CARLE. 1984. Morphology of the metazoan circulatory systems. *Zoomorphology* **103:** 193–208.

SCHOENMAKERS, H., P. COLENBRANDER, J. PEUTE, AND P. VAN OORDT. 1981. Anatomy of the ovaries of the starfish *Asterias rubens.* A histological and ultrastructural study. *Cell Tissue Res.* **217:** 577–598.

SCHROEDER, P. C. 1971. Active contraction of starfish oocyte follicle cells after treatment with 1-methyladenine. *Naturwissenschaften* **58:** 270–271.

SCHROEDER, P. C., J. H. LARSEN, AND A. E. WALDO. 1979. Oocyte-follicle cell relationships in a starfish *Patiria miniata. Cell Tissue Res.* **203:** 249–256.

SCHROEDER, T. E. 1972. The contractile ring II. Determining its brief existance, volumetric changes, and vital role in cleaving *Arbacia* eggs. *J. Cell Biol.* **53:** 419–434.

SCHROEDER, T. E. 1981. Microfilament-mediated surface changes in starfish oocytes in response to 1-methyladenine. Implications for identifying the pathway and receptor sites for maturation inducing hormones. *J. Cell Biol.* **90:** 362–371.

SCHROEDER, T. E., AND J. J. OTTO. 1984. Cyclic assembly-disassembly of cortical microtubules during the maturation and early development of starfish oocytes. *Dev. Biol.* **103:** 493–503.

SMILEY, S. 1984. A description and analysis of the structure and dynamics of the ovary, of ovulation and of oocyte maturation in the sea cucumber *Stichopus californicus.* M.Sc. Thesis Univ. Washington. 119 pp.

STRATHMANN, R. R., AND H. SATOH. 1969. Increased germinal vesicle breakdown in oocytes of the sea cucumber *Parastichopus californicus* induced by starfish radial nerve extract. *Exp. Cell Res.* **54:** 127–129.

TANAKA, Y. 1958. Seasonal changes occurring in the gonad of *Stichopus japonicus. Bull. Fac. Fish. Hokkaido Univ.* **9:** 29–36.

TENNENT, D. H., AND T. ITO. 1941. A study of the oogenesis of *Mespilia globulus. J. Morphol.* **69:** 347–404.

THEEL, H. 1901. On a singular case of hermaphroditism in the holothuroids. *Bihang Svenska Vetensk. Acad. Handl.* **27:** Afd. 4., No. 6.

TYLER, P. A., AND J. D. GAGE. 1983. The reproductive biology of *Ypsilothuria talismani* from the north east Atlantic. *J. Mar. Biol. Assoc. U. K.* **63:** 609–616.

WALKER, C. W. 1982. Nutrition of gametes. Pp. 449–468 in *Echinoderm Nutrition,* M. Jangoux and J. Lawrence, eds. Balkema Press, Rotterdam. 654 pp.

WESSELLS, N., B. SPOONER, J. ASH, M. BRADLEY, M. LUDUENA, E. TAYLOR, J. WRENN, AND K. YAMADA. 1971. Microfilaments in cellular and developmental processes. *Science* **171:** 135–143.

WOOD, R. L., AND M. J. CAVEY. 1981. Ultrastructure of the coelomic lining in the podium of the starfish *Stylasterias forreri. Cell Tissue Res.* **218:** 449–473.